In view of the foregoing, Applicant respectfully requests withdrawal of these objections.

35 U.S.C. § 112 first paragraph Rejection

Claims 19-32 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Specifically, the Examiner states that claim 19 is directed to a method for radiosensitizing cancer cells which includes delivering to cancer cells an effective dose of an expressible nucleic acid molecule encoding a mutant epidermal growth factor receptor (EGFR). Examiner further states that, while a written description for mutant EGFR missing more or less carboxy terminal region (compared with EGFR-CD533) is generally understood, there is no written description regarding different mutants other than a deletion of carboxy terminal region of EGFR that are dominant negative. The Examiner specifically states that there is no written description regarding the "detailed chemical structure" of each type of mutant that is a dominant-negative mutant and possesses the required function for radiosensitizing cancer cells.

Applicant's have added new claim 33 which recites a method for radiosensitizing cancer cells by delivering to the cancer cells an effective dose of an expressible nucleic acid encoding a carboxy terminal truncated mutant epidermal growth factor. (Applicant notes that the issue of "radiosensitizing" in new claim 33 is further addressed below.) Applicant submits that, as indicated by Examiner, the subject matter of new claim 33 is clearly supported by the specification.

Other mutant forms

With respect to Examiner's comments concerning support for other mutant forms of EGFR, Applicant has hereby amended Claim 19 to recite that the "mutant" is a "dominant negative mutant" of EGFR. Dependent claim 20 has likewise been amended to conform to the language of amended claim 19. "Dominant negative mutant" is a term of art that refers to a mutant form of a protein, the expression of which serves to counteract or negate the phenotypic function of the corresponding wild type protein. EGFR-CD533 represents a dominant negative form of EGFR since its expression, while not directly interfering with transcription and translation of the wild type gene, renders the function

of the wild type protein moot. It is believed that the EGFR-CD533 protein dimerizes with wild type EGFR and prevents normal activation of EGFR. This is advantageous in situations where it is desirable to prevent EGFR activity, such as when treating cancer cells with radiation. Radiation has been shown to activate EGFR and lead to the phenomenon of "accelerated repopulation" of cancer cells, so that those cancer cells not destroyed by radiation may actually multiply more rapidly after exposure to radiation than before. This is clearly an undesirable outcome.

In one aspect, the present invention provides a means to prevent accelerated repopulation of cancer cells during radiation therapy by administering a dominant negative mutant form of EGFR. EGFR-CD533, in which 533 carboxy terminal amino acids have been deleted from EGFR, is one example of such a dominant negative mutant. As recognized by Examiner, other carboxy terminal truncations which are not identical to that of EGFR-CD533 would be predicted to have similar dominant negative properties. However, Applicant submits that those of skill in the art would recognize that dominant negative mutants may arise or be developed by strategies other than truncating carboxy terminal amino acids. For example, CD533 encompasses the first 671 amino acids of EGFR, including the extracellular domain and the transmembrane domain spanning the isoleucine at position 622 to the methionine at position 644 (I622-M644), but lacking the cytoplasmic protein kinase domain. Thus, the critical tyrosine phosphorylation sites (amino acid positions 845, 974, 992, 1045, 1068, 1086, 1101, 1148 and 1173) are absent. These tyrosine residues are important for interacting with SH2 binding proteins (e.g. Grb2), and it is likely that results similar to those obtained with the truncated CD533 mutant would be obtained with a mutant in which all these tyrosine residues had been either individually deleted, or substituted with a non-phosphorylatable amino acid such as alanine. Further, those of skill in the art will recognize that other deletions within the cytoplasmic domain, perhaps less extensive than that of CD533 but also eliminating many or all of the tyrosine residues, would also be predicted to display the dominant negative phenotype and function similarly to CD533. Applicant submits that any dominant negative mutant constructed by mutation of the cytoplasmic domain would be predicted to display properties similar to those of CD533, and therefore be useful in the practice of the present invention. Applicant has therefore hereby amended claim 19 to recite that the method encompasses delivering an effective dose of an expressible nucleic acid encoding a dominant negative mutant epidermal growth factor.

In view of the foregoing, Applicant requests withdrawal of this portion of this rejection.

Claims 19-32 have also been rejected under 35 U.S.C. 112, first paragraph, because in the opinion of the Examiner, while the specification is enabling for "directly delivering to cancer cells an effective dose of an expressible nucleic acid molecule encoding a carboxy terminal truncated EGFR for inhibiting the radiation-induced proliferation of cancer cells", does not reasonably provide enablement for 1) delivering to cancer cells an effective dose by other routes, or 2) radiosensitizing cancer cells.

Applicants have hereby added new claim 34. New claim 34 is directed to directly delivering to cancer cells an effective dose of an expressible nucleic acid molecule encoding a carboxy terminal truncated mutant of EGFR for inhibiting the radiation-induced proliferations of cancer cells.

However, Applicant respectfully disagrees with Examiner's assessment regarding enablement for the broader subject matter of claim 19.

Methods of delivery

With respect to methods of delivery of the mutant EGFR, Examiner states that the specification"does not provide any teaching or guidance as how to deliver effective amount of Ad-EGFR-CD533 or EGFR-CD533 by any other delivery vectors...". Applicant respectfully disagrees. On page 17 at lines 28-30 and continuing on page 18, lines 1-17, alternate means of delivery, including other types of vectors, are described. Further, specific modifications of the adenoviral vector used in Ad-EGFR-CD533 are described on page 18 at lines 18-29.

Applicant submits that the field of gene therapy has progressed significantly and that a body of literature which provides guidance to those in the field exists. For example, Applicant's herewith provide copies of the following articles which discuss the success of various types of systems for the delivery of nucleic acids to cells: Gokhale et al, which describes delivery via cationic liposomes; Printz et al, which describes the use of alternative adenoviral vectors; and Xu et al, which discusses transferrin-liposome mediated gene therapy. Applicant submits that those of skill in the art will recognize that the means of delivery of the genetic construct of the present invention is not a key feature of the invention, and that modifications to the vector would have no bearing on the outcome

of the therapy so long as the resulting construct is delivered to the cancer cells. Rather, the point is to deliver the construct to the cancer cells so that it is expressed, whatever the method of delivery might be, and whatever the precise design of the vector construct. Applicant submits that a skilled artisan, such as a physician well-versed in gene therapy, will be aware of the various alternatives available for such delivery (for example, those described in the enclosed publications), and would require no more explicit teaching than that which is found in the present application in order to successfully practice the present invention.

In view of the foregoing, Applicant requests withdrawal of this portion of this rejection.

Radiosensitization

With respect to the radiosensitization of cancer cells by delivery of a mutant EGFR, Examiner states that "These data do not suggest that decreased tumor survival rate is resulted from a direct radiosensitization of cancer cells. There is no evidence that decrease tumor survival rate is a result of increased killing of tumor cells by radiation." Applicant strongly disagrees with Examiner's statements.

"Radiosensitization" is defined in the specification of the application at page 13 at lines 22-24, where it is stated: "The term 'radiosensitize,' when used in reference to a tumor or a tumor cell, means to increase susceptibility of the tumor or tumor cell to the effects of radiation. The term 'radiosensitize' is used in a comparative sense and, with regard to the present invention, indicates that the radiation dose to reduce the severity of a cancer in a subject that has been treated as disclosed herein is less than the radiation dose that would have been required if the subject had not been treated." "Accelerated proliferation" is defined on page 14 at lines 12-13: "The term 'accelerated proliferation' or 'accelerated repopulation' refers to the radiation-induced proliferation of surviving tumor cells after ionization." That the invention deals with both phenomena is evidenced by the discussion presented on page 15, at lines 9-14, which states: "The present invention is based on the discovery that the delivery of DNA encoding a mutant, dominant negative form of EGFR to tumor cells causes radiosensitization of tumor cells, and that this radiosensitization is a *two-fold phenomenon*. On the one hand, direct radiosensitization of tumor cells in general is provided. In addition, the methods of the present invention overcome the phenomenon of accelerated repopulation

by inhibiting the proliferative capacity of tumor cells which develops in response to radiation." (emphasis added) ; and at lines 24-26, which state: "Accordingly, the present invention provides a method for 1) directly radiosensitizing cancer cells to radiation, and 2) suppressing the phenomenon of accelerated repopulation in cancer cells of a patient undergoing radiation therapy."

Examiner has agreed that the data presented demonstrates the suppression of accelerated repopulation. Applicant submits that direct radiosensitization of cancer cells is also amply demonstrated in a manner that is art-recognized.

In particular, Example 3 describes experiments conducted with human mammary carcinoma cells *in vitro* and *in vivo*. *In vitro* radiation studies were carried out and analyzed as described in Materials and Methods. In particular, radiosensitization data was analyzed by the art-recognized method by Fertil et al, 1984, which is referenced on page 38, at lines 27-28 of the present application. A copy of Fertil et al. is enclosed for Examiner's convenience. The method involves computation of " $\overline{\mathbf{D}}$ ratios" which are then used to calculate art-recognized "dose enhancement ratios" (see page 40, lines 14-16 of the present application). Dose enhancement ratios are recognized by those of skill in relevant arts as measures of radiosensitization. Further, data which is used to assess radiosensitization is typically (although not exclusively) acquired after a single exposure to radiation, where is it possible to compare cell survival of experimental and control cells prior to the onset of accelerated repopulation. In contrast, accelerated repopulation data is mostly observed after repeated radiation exposures (see page 42, lines 3-6 of the present application). The results obtained in the present application clearly address this art-recognized distinction. For example, on page 41, lines 19-30 and continuing on page 42, lines 1-6 of the present application, a succinct account of experiments that show radiosensitization is given. The data is presented in Figure 17, and is summarized in the text (page 42, lines 3-6) as follows: "This data demonstrates that over-expression of EGFR-CD533 results in direct radiosensitization of MDA cells after single radiation exposures in addition to inhibition of the previously described radiation-induced proliferation response that is most impressively shown after repeated radiation exposures (Contessa et al., 1999)" (underline added). Thus the data presented (e.g. in Figure 17) reflects radiosensitization of cancer cells, rather than accelerated repopulation. Further experiments proving radiosensitization are presented in the single dose radiation experiment described in Example 4 on page 50 at lines 7-18, and in Figure 32.

On lines 16-18, it is stated that: "This reduced clonogenic survival for cells expressing EGFR-CD533 was similar in the U-373 MG cells with a 38% survival reduction after a single dose of 4 Gy ($P < 0.0001$; Figure 32)."

Applicant further notes that the material presented in Examples 3 and 4 has now been published in peer-reviewed journals (Example 3 as "Epidermal Growth Factor Receptor as a Genetic Therapy Target for Carcinoma Cell Radiosensitization"[underline added], *Journal of the National Cancer Institute*, 2001; 93:921-929; and Example 4, as "Radiosensitization of Malignant Glioma Cells through Overexpression of Dominant-Negative Epidermal Growth Factor Receptor" [underline added], *Clinical Cancer Research*, 2001, 7:682-690). Copies of these publications are enclosed for the convenience of Examiner. In both cases, the data was accepted by experts in the field as demonstrating radiosensitization of cancer cells, as indicated by the title and the material presented within.

Applicant thus submits that the results presented in the present application clearly demonstrate direct radiosensitization of cancer cells.

In view of the foregoing, Applicant respectfully requests withdrawal of this portion of the rejection.

Use of nude mouse model

The in vivo studies described in the present invention utilized athymic nude mice in a nude mouse/clonogenic assay. Examiner has stated that the use of the nude mouse model is insufficient, and that "the art teaches that the growth of human tumors in immunocompromised mice does not reflect the natural and physiological growth and spread of tumors in non-immunocompromised animals, and cites a single reference by Gura as evidence of "the art"as a whole. Applicant disagrees with Examiner's assessment of "the art".

On the contrary, in the field of cancer therapy development, the nude mouse model is accepted as the gold standard for identifying compounds and/or procedures that show promise for human clinical trials, a decision which is not taken lightly by those in the field. Applicant herewith submits a publication by Martin et al. (1986, copy enclosed) which directly speaks to this point. In the article, the "disenchantment" with murine tumor models is discussed, and arguments are presented as to why disenchantment is unwarranted. The article concludes that, in the real world

where no therapeutic approach is unequivocal, the murine tumor model is extremely valuable in optimizing dosage and administration schedules, and in the development of new drugs.

Similarly, an article by Winograd et al, (1987, copy enclosed) describes the "good correlation of drug effects in the nude mouse model"(see abstract). Fiebig et al (1984, copy enclosed) states that "...the highly correct prediction rates for tumor sensitivity and resistence validates human tumor xenografts as tumor models to test new drugs and combinations"(see abstract). Finally, Berger et al. (1990, copy enclosed) speaks directly to the use of human tumor xenografts and their predictive value. The study shows excellent correlation between clinical efficacy of known anticancer agents, and concludes that "A combined approach (nude mouse/clonogenic assay) [such as that utilized in the present invention] should find increases application as a screen for new anticancer drugs." (last sentence of abstract).

Applicant submits that variability in response to drugs of any kind is inherent in medical treatments of any kind. There are no "magic bullets", particularly for cancer. Even established therapies (e.g. the use of cisplatin) can be more or less efficacious depending on many factors, some of which are not known with certainty. Nevertheless, cisplatin is still a treatment of choice.

Applicant submits that the therapeutic agent of the present invention performed exceedingly well in the art-accepted model system and can be accurately described as an claimed as an agent for bringing about radiosensitization of cancer cells and blocking of accelerated repopulation.

In view of the foregoing, Applicant respectfully requests withdrawal of this portion of the rejection.

35 U.S.C. § 102(a) Rejection

Claims 19-23, 25, 29 and 32 stand rejected under 35 U.S.C. § 102(a) as being anticipated by Contessa et al,. *Clinical Cancer Research* 5:405-11, 1999. Examiner states that Contessa et al, teach methods of construction of cell lines which contain EGFR-CD533 that is inducibly expressed, and that EGFR-CD533 may be used therapeutically via transduction of carcinoma cells.

Applicant herewith encloses a declaration under 37 CFR 1.131, together with supporting evidence, which shows that the inventors Schmidt-Ullrich and Valerie were in possession of the subject matter of the present invention prior to the date of publication of the Contessa et al.,

reference (February 1999). The supporting material includes a copy of the original manuscript for the Contessa et al. publication as submitted and reviewed by the editors, a copy of the letter of submission, and a declaration by the inventors. Examiner will note that the letter of submission of the manuscript is dated June 24, 1998, and that in the lower left hand corner of the front page of the manuscript, there is a hand-written note that the manuscript was received on June 26, 1998. The inventors of the present invention, Dr. Rupert Schmidt-Ullrich and Dr. Kris Valerie, are listed as joint authors of the manuscript. The manuscript contains all of the experimental data and discussion that was eventually presented in the publication. This includes a description of the transduction of carcinoma cells with the EGFR-CD533 genetic construct, and the resulting radiosentization of the cancer cells. As indicted in point number 5 of the declaration, the abstract of the manuscript (last three sentences) states that "...repeated 2 Gy exposures of [cancer] cells, under conditions of EGFR-CD533 expression, essentially abolished their ability of subsequent growth. *These results identify the inhibition of EGFR function through genetic manipulation as a potential therapeutic maneuver.* The concept of such an intervention would be the *radiosensitization* of [cancer] cells by counteracting a radiation-induced cytoprotective proliferation response." (italics added for emphasis). The publication further states the following on page 14, beginning on the second sentence of the last paragraph and continuing on page 15, lines 1-3, "These promising results of enhanced radiation toxicity strongly suggest that the genetic manipulations used can be exploited therapeutically. Such a therapeutic application could be accomplished through efficient transient transduction of carcinoma cells using recombinant adenovirus containing the EGFR-CD533 gene...". Thus, the inventive concept of radiosensitizing cancer cells by delivering to the cancer cells an effective dose of an expressible nucleic acid molecule encoding a dominant negative mutant epidermal growth factor receptor, as recited in claim 19 of the present application, is directly stated in the manuscript. Further, the manuscript, and thus the inventive concept discussed therein, was in existence at least by the date of submission of the manuscript (June 24, 1998), and predates the reference to Contessa et al (February of 1999).

Applicant further encloses copies of declarations from each of the co-authors of the Contessa et al. reference. In the declarations, each co-author declares that he/she has reviewed the present patent application, and is not an inventor of the claimed subject matter.

In view of the foregoing, Applicant respectfully requests withdrawal of this rejection.

35 U.S.C. § 103(a) Rejection

Claims 19-25 and 27-32 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over a combination of Greene et al (U.S. patent 6,417,168) and Contessa et al. 1999.

Examiner states that Greene et al. teach methods of treating individuals with erbB protein mediated tumors by administering nucleic acid molecules that encode a protein the dimerizes with the erbB protein. Examiner further states, however, that Greene et al. does not explicitly teach the use of a nucleic acid encoding C-terminal deletions of EGFR for such a treatment, but that Contessa et al. supplies this element and that the combination of the two references therefore renders the present invention obvious.

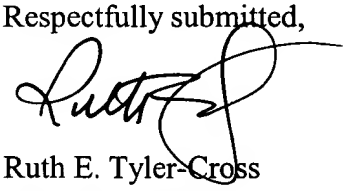
Applicant respectfully submits that, as discussed above regarding the 35 U.S.C. § 102(a) rejection based on Contessa et al., Contessa et al. is not available as a prior art reference against the present application. Therefore, Contessa et al. cannot be combined with any other reference to render the present invention obvious.

In view of the foregoing, Applicant respectfully requests withdrawal of this rejection.

Closing Remarks

In view of the above, Applicant submits that claims 19-35 should be deemed new and unobvious over the prior art of record. Reconsideration and allowance of the claims at an early date is requested.

Respectfully submitted,



Ruth E. Tyler-Cross
Reg. No. 45,922

703-787-9400
Whitham, Curtis & Christofferson
11491 Sunset Hills Road
Reston, VA 20190

30743
PATENT TRADEMARK OFFICE